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(71) Sökande Applicant (s) — *Sahltech i Göteborg AB, Göteborg SE*

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Åsa Dahlberg

Åsa Dahlberg

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USE OF INTERLEUKIN-6 IN TREATMENT OF OBESITY AND DISTURBANCES OF BLOOD FATS

Technical field of the invention

The present invention relates to a new medicinal product and a new method for treatment of pathological disturbances of regulation of body fat tissue mass and blood fat concentrations.

Background art

Obesity in humans and mice

Obesity is a large problem in the Western world since it is associated with increased mortality. Generally, obesity is due to energy intake that greater than energy expenditure. This can be caused by overeating, i.e. higher food intake than necessary for maintenance of body mass. In addition, low mobility and low metabolic rate may predispose for obesity (see Flier, J. S. and Foster D. W. (1998) Eating disorders: obesity, anorexia nervosa, and bulimia nervosa. In: Williams Textbook of Endocrinology, 9th Ed, Saunders Co.). Animal models can be used for investigation of which genes that are causing development of obesity. Of particular importance is the information that can be gained from mouse strains that develop obesity because of gene knockouts. These mouse strains can provide evidence that a certain gene product is of crucial importance for regulation of body fat. This in turn may facilitate the development of new treatment paradigms. There are indications that there are gender differences regarding the genetic ethiology of obesity (see e.g. Costet, P. et al. (1998) Peroxisome Proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J. Biochem. Chem. 273,29577-29585).

Obesity and blood fats in relation to cardiovascular disease

It is recognized that obesity, especially visceral obesity, and deranged lipid-lipoprotein profile, including hypertriglyceridemia and hypercholesteolemia are associated with larger risk of cardiovascular disease (Lamarche B, et al. (1998), Visceral obesity and the risk of ischemic heart disease: insights from the Quebec cardiovascular study. Growth hormone and IGF research 8, (suppl. B) 1-8.). So far, a lot of the research on the ethiology of this syndrome has dealt with neuroendocrine, i.e. hypothalamohypophyseal, and endocrine disturbances, focusing on the effects of the hypothalamus-pituitary-adrenal (HPA) axis regulating glucocorticoid, sex steroids and growth hormone (see e.g. Björntorp, P. (1996) The regulation of adipose tissue distribution in humans, Int. J. Obesity 20, 291-301.)

Leptin and obesity

Leptin is produced by adipose tissue and the levels of leptin in circulation essentially reflect the fat tissue mass. Leptin is thought to participate in a negative feedback regulation of body fat mass via effects on the hypothalamus. Following the cloning of leptin 5 years ago (see Zhang et al. (1994), Positional cloning of the mouse *ob (obesity)* gene and its human homologue. Nature 372, 425-432), there were great hopes that this would mean new possibilities to treat obesity and overeating. However, later it was found that obesity in humans very seldom is due to leptin deficiency, but rather associated with increased leptin levels. Moreover, it has been shown that both mice and humans often are resistant to the anti-obesity effect of leptin (see e.g. Flyer, J. S. (1998), What's in a name? In search of leptin's physiological role, J Clin. Endocr. Metab 83, 1407-1413, and references therein).

Structures of interleukin-6 and its receptor

Interleukin-6 (IL-6) exerts its biological effects through the ligand-specific IL-6 receptor, which belongs to the cytokine receptor superfamily. The multisubunit IL-6 receptor complex consists of the IL-6Rα subunit which binds to IL-6 and the membrane associated glycoprotein gp130 which is a signal transducer. Unlike most other cytokine receptors, the IL-6Rα subunit can be activated by ligand binding in both its membrane bound and its soluble form. IL-6 induces heterodimerization between IL-6Rα and gp130, which in turn leads to homodimerization of gp130 to a second gp130 molecule (see e.g. Hirano, T. (1998), Interleukin 6 and its receptor: ten years later. Int. Rev. Immunol. 16, 249-284). Actually, IL-6/IL-6Rα complexes can be potent activators of gp130, including in cells that lack membrane bound IL-6Rα. Since gp130 can be activated by several other ligand-receptor complexes, these effects may not reflect the physiological role of IL-6 (see e.g. Schirmacher, P., et al. (1998), Hepatocellular hyperplasia, plasmacytoma formation, and extramedullary hematopoiesis in interleukin (IL)-6/soluble IL-6 receptor double-transgenic mice. Am. J. Pathol. 153, 639-648). On the other hand, the fact that several different types of cytokine receptors can activate gp130 opens the possibility that different cytokines may potentiate each others actions thereby exerting synergistic effects. One example of receptors belonging to the IL-6Rα family is the leptin receptor (Tartaglia, L. A. et al., (1995), Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263-1271) but the leptin receptor is not acting via gp130 (see e.g. Baumann, H., (1996), The full-length leptin receptor has signaling capabilities of interleukin 6-type receptors. Proc Natl. Acad. Sci. USA 93, 8374-8378).

Most patents issued regarding IL-6 have described methods to get beneficial effects of suppression of IL-6

action. One exception is a recent patent claiming that IL-6 can suppress demyelination, e.g. during multiple sclerosis (see US Pat. No. 5,863,529) Methods have been developed for production of human IL-6 in large quantities (see e.g. US Pat. No. 5,641,868).

IL-6 and acute phase reaction (APR)

IL-6 plays a role for different parts of the immune response (see e.g. Hirano, T. (1998), supra). It is well known that production of IL-6 as well as the circulating levels of this cytokine is enhanced during so called acute phase reaction (APR). Moreover, IL-6 is considered a key mediator of APR, especially after infection with gram positive bacteria (see e.g. Kopf, M., et al. (1994), Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368, 339-342). The APR is characterized by changes in the composition of the proteins released into plasma from the liver. APR is seen in pathological conditions with an inflammatory component such as trauma, infections, autoimmune disease, and tumors. These conditions are also associated with catabolism, i.e. decreased growth and increased degradation of tissues belonging to the fat free mass in the body.

IL-6 and ageing

Ageing is associated with several somatic changes including increased fat body mass in general and visceral fat mass in particular (see e.g. Rudman, D., et al., (1990), Effects of human growth hormone in men over 60 years old. N. Engl. J. Med. 323, 1-6; Flier, J. S. and Foster D. W. (1998) supra). The proportion of the population that have disturbances of blood fats such as pathologically elevated serum triglycerides also increase with age and is higher in middle aged than in young adult persons (Brown, M. S., and Goldstein, J. L. (1983) Disorders of lipid metabolism, Harrison's principle of internal medicine, 10th Ed, 547-559. It has been suggested that

several age-associated diseases are caused by enhanced IL-6 (see e.g. Ershler, W. B., et al., (1994), The role of interleukin-6 in certain age-related diseases. Drugs Aging 5, 358-365). In humans there is an epidemiological connection between high IL-6 levels in peripheral blood mononuclear cells (PBMC) (see e.g. O'Mahony, L., et al., (1998), Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin. Exp. Immunol. 113, 213-219) as well as in serum (see e.g. Mysliwska, J., et al., (1998), Increase of interleukin 6 and decrease of interleukin 2 production during the aging process are influenced by the health status. Mech. Aging Dev. 100, 313-328).

Effects of low, normal levels of IL-6 in mice of different age

There is much information about the effects of high levels of IL-6, e.g. in connection with inflammation (see e.g. Kopf, M., et al, supra). However, little is known about the importance of the low, basal levels of IL-6 in animals and humans without inflammation. One reason could be that it has been difficult to measure the low IL-6 levels in healthy mice with the assays available today. However, it can not be excluded that there still is a biologically significant effect of IL-6 in these animals. Moreover, IL-6 that is produced in local tissues may exert autocrine or paracrine effects on cells in the same tissue, without being transported to other organs via blood circulation.

There have been few reports of differences between mice with complete IL-6 deficiency due to targeted disruption of the IL-6 gene, and normal wild type mice in the absence of provocations (see e.g. Hirano, T. (1998), supra). It is known that these mice develop normally to adulthood and they are fertile (see e.g. Kopf, M., et al, supra, and Poli, V., et al., (1994). Interleukin-6 deficient mice have been reported to be protected from bone

loss caused by estrogen depletion. EMBO J. 13, 1189-1196). It has also been reported that IL-6 mice might have a defective fever response (see e.g. Hirano, T. (1998), supra). However, very little has been published about the effects of IL-6 deficiency in mice that are older than a couple of months. This could be due to the fact that it is expensive and laborious to keep mice for longer time. Since the normal life span of a mouse is two years, there are few publications about a large part of the adult life of mice.

Regulation of IL-6 production and release

As mentioned above, IL-6 is released during acute phase reaction. Therefore, it is not surprising that IL-6 production is enhanced by gram-positive as well as by gram-negative bacteria. The latter seem to release IL-6 via production of an antigen called lipopolysaccharide (LPS) (see e.g. Kopf, M., et al. (1994), supra). The production of IL-6 is enhanced by tumor necrosis factor-α, TNF-α, a cytokine thought to play a role for induction of type 2 diabetes, an illness associated with visceral obesity and cardiovascular disease. TNF-α production is enhanced from adipocytes that have accumulated fat (see e.g. Hotamisligil G. S. and Spiegelman B. M., (1994), Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 43, 1271-1278; Flier, J. S. and Foster D. W. (1998), supra).

Several other hormones have also been shown to enhance IL-6 production. These include parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3, thyroid hormone, platelet-derived growth factor, insulin-like growth factor I, and IL-1 (see e.g. Swolin, D., et al., (1996), Growth hormone increases interleukin-6 produced by human osteoblast-like cells. J. Clin. Endocrinol. Metab. 81, 4329-4333, and references therein). It has also been reported that corticosteroids, which are well known inducers of visceral obesity, can suppress IL-6 expression (see e.g.

Swolin-Eide, D., et al., (1998), Effects of cortisol on the expression of interleukin-6 and interleukin-1 beta in human osteoblast-like cells. J. Endocrinol. 156, 107-114).

IL-6 and body fat during APR

IL-6 is a major mediator of APR, a condition associated with wasting and decreased appetite. However, it is still by no means certain that IL-6 also causes these anorectic and wasting effects. In fact, there are data indicating that this is not the case, although lipopolysaccharides (LPS) were reported to induce weight loss in mice and that this effect can be significantly prevented by treatment with anti-IL-6 monoclonal antibodies. However, in the same study the anti-IL-6 antibodies did not prevent the hypertriglyceridemia induced by LPS, possibly suggesting that IL-6 is less important for changes in fat metabolism during APR (Strassman, G. et al. (1993), The role of interleukin-6 in lipopolysaccharide-induced weight loss, hyperglycemia and fibrinogenproduction. Cytokine 5, 285-290).

It has been reported that IL-6 treatment can decrease lipoprotein lipase (LPL) activity in adipose tissue of mice and in murine adipocyte cell lines in vitro. This effect has been seen as an indication of a lipolytic effect of IL-6 during cancer cachexia, a condition associated with APR (see Greenberg, A. S., (1992), Interleukin-6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin-6 in cancer cachexia, Cancer Res. 52, 4113-4116). On the other hand, there are indications e.g. from studies of gene knockout mice that LPL activity does not affect fat accumulation (Zechner, R (1997), The tissue specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism, Curr. Oppin. Lipidol. 877-88).

IL-6 and body fat during normal conditions

It has been speculated that that IL-6, like leptin, could have an adipostatic activity also in patients without APR. However, this assumption was based only on the finding that subcutaneous fat releases IL-6 in patients without acute phase reaction. Not surprisingly, there was also a correlation between high BMI, presumably reflecting fat mass, and levels of circulating IL-6 (Mohamed-Ali, V., et al. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo, J. Clin. Endocrinol. Metab. 82,4196-4200). However, the finding that IL-6 is released by adipose tissue, does in no way prove that this factor would regulate fat tissue mass. As noted above, it is by no means clear that IL-6 is of importance for lipolysis even during APR. In the absence of APR, the available data has suggested that long term treatment with IL-6 in low, physiological doses is not lipolytic by itself. Although a single injection of IL-6 in a dose of 50 µg/kg body weight has been shown to enhance release of free fatty acids into blood circulation (Nonogagi K, et al. (1995), Interelukin-6 stimulates hepatic triglyceride secretion in rats, Endocrinology 136, 2143-2149), there is no obvious loss of fat mass in transgenic mice with very high levels of circulating IL-6 (see e.g. Peters, M. (1997), Extramedullary expansion of hematopoietic progenitor cells in interleukin (IL)-6-sIL-6R double transgenic mice. J. Exp. Med. 185,755-766), although such mice display growth impairment (De Benedetti, F. et al. (1997), Interleukin-6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-1. J. Clin. Invest. 99, 643-650) as well as muscle atrophy (Tsujinak, T et al. (1996), Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. J. Clin. Invest. 97, 244-249). Moreover, there have been few indications in the literature that long term absence of the low physiological

amounts of endogenous IL-6 that are produced in an animal or human without APR, would have consequences for fat metabolism, especially fat mass and blood fat levels. The best way to investigate the consequences of long term absence is probably the study of mice with IL-6 gene knock out. In 1998 one of the worlds leading experts on IL-6 concluded in a review the results of IL-6 knock out mice had shown "that IL-6 is critical in only a limited range of biological reactions such as APR, the mucosal IgA response, the fever response, and estrogen deficiency-induced bone loss." (see e.g. Hirano, T. (1998), supra, p 252). No effects of fat mass in IL-6 knock-out mice have been reported. As noted above, IL-6 can suppress LPL (see Greenberg, A. S., (1992), supra), and it has also been suggested that LPL can increase predisposition for obesity and fat accumulation. On the other hand, this theory is challenged by the fact that fat specific deltion LPL activity does not affect fat mass (Zechner, R (1997), The tissue specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism, Curr. Oppin. Lipidol. 8,77-88). The general opinion by well reknowned researchers today is that IL-6 does not affect fat mass essentially, especially not during normal life without APR.

IL-6 and ethanol

Under certain circumstances, alcohol can suppress the concentration of circulating IL-6 (see e.g. Akerman, P. A., et al. (1993), Long-term ethanol consumption alters the hepatic response to the regenerative effects of tumor necrosis factor-alpha. Hepatology 17, 1066-1073). It is also well known that ethanol can cause visceral obesity as well as deranged blood fats including enhanced serum triglyceride levels (Brown, M. S., and Goldstein, J. L. (1983), supra).

TNF-α and regulation of body fat

As mentioned above, TNF-α is a stimulator of IL-6 production. This effect of TNF-α is exerted via the type 1 (p55) receptor, since it has been shown that IL-6 levels are decreased in mice with TNF receptor 1, but not TNF receptor 2, gene knock out (Yamada, Y., et al. (1998), Analysis of liver regeneration in mice lacking type 1 or type 2 tumour necrosis factor receptor: requirement for type 1 but not type 2 receptor. Hepatology 28,959-970). The role of TNF-α for development of obesity is not clear. Mice lacking the TNF-α ligand have been reported to be obese (Uysal, K. T., et al (1997), Protection from obesity induced insulin resistance in mice lacking TNF-α, Nature 389,610-614), while there was no obesity in mice deficient in the both of the two receptors, type 1 (p55) and type 2 (p75), that are thought to mediate the biological effects of TNF-α. Actually, mice deficient in the type 2 (p75) receptor gain less weight when given high fat diet, suggesting that TNF-α might even stimulate obesity via this receptor type (Schreyer, S. A. (1998), Obesity and diabetes in TNF-α receptor deficient mice. J. Clin. Invest. 102,402-411). Furthermore, no increase in body weight was found in mice with TNF receptor 1 gene knock out even when they were fed high fat diet (Schreyer, S. A. (1998), supra). Obesity in *db/db (diabetes/diabetes)* mice with a defective leptin receptor, was not affected by lack of the TNF receptor 1 (Schreyer, S. A. et al (1998), supra) or by lack of the ligand TNF-α which activates both receptor 1 and receptor 2 (Uysal, K. T., et al., (1997), supra). Another finding that argues against beneficial effects of TNF-α in obesity is that TNF -α often enhances insulin resistance, a symptom often associated with obesity (see Flier, J. S. and Foster D. W. (1998), supra).

Cytokines and atherosclerosis

Although the interest in the possible associations between cytokines and atherosclerosis has increased during recent years, it has mostly concerned the possible deleterious effects of cytokines and inflammation in development of atherosclerosis The cytokines have been assumed to stimulate the development of the atherosclerotic plaques by local effects (see e.g. Rus, H. G., et al., (1996) Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis 127,263-271). In addition, as mentioned above, IL-6 has been reported to increase circulating triglycerides by release of triglycerides from the liver (Nonogagi K, et al. (1995), Interleukin-6 stimulates hepatic triglyceride secretion in rats, Endocrinology 136, 2143-2149).

Summary of the invention

The object of the present invention is to provide new medicinal products and methods for treatment of conditions associated with obesity and disturbances of blood fats.

The invention relates to the use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for the production of a medicinal product for the treatment of obesity and/or disturbances of blood fats.

Furthermore, the invention relates to a method for treatment of obesity and/or disturbances of blood fats wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient.

The characterizing features of the invention will be evident from the following description and the appended claims.

Detailed description of the invention

In the research work leading to the present invention it was found that endogenous IL-6 can inhibit development of "middle-aged"-onset obesity as well as disturbances of blood fats, i.e. conditions that i.a. are related to cardiovascular diseases in the western world.

The invention thus relates to medicinal products comprising a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist. Said substance may be an IL-6 receptor agonist. A preferred example of such an agonist is IL-6. It is possible to use a naturally occurring agonist, such as IL-6, as well as a synthetically produced agonist, such as an IL-6 mimetic. Said substance may also be a substance that upon administration will lead to the release of an endogenous occurring IL-6 receptor agonist, preferably IL-6, from different cells, such as endothelial cells, or organs, such as the liver.

The expression "IL-6 receptor agonist" used herein relates to all substances that bind to and activate the same receptor proteins as IL-6.

The term "patient" used herein relates to any human or non-human mammal in need of treatment with the medicinal product or method according to the invention.

The term "treatment" used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The treatment may either be performed in an acute or in a chronic way.

As mentioned above, the invention is suitable for treatment of disturbances of blood fats. Examples of such disturbances are high levels of triglycerides or high levels of cholesterol. The expressions "high levels of triglycerides" and "high levels of cholesterol" relates to amounts of these compounds that are higher than for a normal, healthy person.

The medicinal product and the method according to the invention are suitable for treatment of different pathological disturbances of regulation of body fat tissues and blood fats, leading to obesity or disturbances of blood fats. One example of such a disturbance is visceral or general obesity that is de to genetic predisposition, a condition sometimes described as the thrifty genotype. Another example is diet-induced obesity, a condition that often is resistant to leptin treatment.

The medicinal product and the method according to the invention are e.g. suitable for treatment of cardiovascular disease, since obesity and disturbances of blood fats are associated with an increased risk of cardiovascular disease.

The medicinal product and the method according to the invention are also suitable for treatment of persons that have been exposed to high doses of glucocorticoid hormone, e.g. due to tumours producing such hormones, due to treatment with glucocorticoids against certain diseases, or due to abuse of glucocorticoids. It is known that high levels of glucocorticoids cause visceral obesity and disturbed blood fats. It has been shown that glucocorticoids under certain circumstances can decrease IL-6 production.

Other patients which may be treated with the medicinal product or the method according to the invention are persons with obesity, blood fat disturbances, and low endogenous production of IL-6 during normal state, i.e., in the absence of APR. Also persons with obesity and blood fat disturbances in combination with insensitivity to IL-6 may be treated with the medicinal product and the method according to the invention. The IL-6 insensitivity could e.g. be caused by low levels of the receptor protein IL-6Rα on the cell surface or low levels of the glycoprotein gp130 which normally mediates the effects of IL-6. In these persons, the IL-6 produced by the patients

themselves may not be sufficient to inhibit development of obesity and blood fat disturbances.

Another example of a group of patient which may be treated according to the invention are patients suffering from during normal aging. In some cases, the production of IL-6 in important tissues could be insufficient although the circulating levels often are increased. A possible IL-6 insufficiency in aging may also be due in part to insensitivity to IL-6.

It is also possible to treat patients with obesity and blood fat disturbances in combination with low concentrations of growth hormone (GH) receptors or defective GH receptors. It is known that GH has lipolytic effects.

It is also possible to treat obese patients with low concentrations of leptin or leptin receptors, or patients with defective leptin receptors. More often, it would be beneficial to treat patients with obesity and blood fat disturbances in combination with leptin resistance due to unknown reasons.

Also patients abusing alcohol may suffer from conditions treatable according to the present invention. It has been shown that alcohol may decrease IL-6 levels (Akerman, P. A., et al. (1993), supra) and that patients abusing alcohol often display increase visceral obesity and enhanced serum triglyceride levels in man (Flier, J. S. and Foster D. W. (1998), supra).

It may be advantageous to combine the substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist used according to the invention with a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist, and the medicinal product according to the invention may thus also comprise such a factor. An example of such a factor is a soluble IL-6 binding protein. However, a problem may be that IL-6 in combination with soluble IL-6Rα may exert unspecific effects, including

even on cells that do not have membrane bound IL-6Rα (see e.g. Peters, M. (1997), supra).

The medicinal product according to the invention may also comprise other substances, such as an inert vehicle, or pharmaceutical acceptable adjuvants, carriers, preservatives etc., which are well known to persons skilled in the art.

The medicinal product according to the invention may be formulated for oral or parenteral administration.

The invention also relates to use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for a medicinal product for treatment of the above specified conditions.

Furthermore, the invention relates to a method for treatment of pathological disturbances of fat metabolism wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient. Preferably, said substance is administered together with a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist.

Since these effects of IL-6 on fat metabolism were first seen in the work leading to the present invention after removal of endogenous IL-6, it seems appropriate to use IL-6 according to the invention in doses that previously have been used to substitute for IL-6 deficiency. Such a dose would be about 1 mg/kg body weight given as a subcutaneous injection to mice (se e.g. e.g. Cressman, D. E., et al., (1996), Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274, 1379-1383). However, the dose of IL-6 in humans could be quite different. The dose may be higher in older individuals, since it has been shown that IL-6 levels increase with age. The dose may be lower those doses that

would result in IL-6 levels found during APR, to avoid side effects similar to the symptoms of APR.

The invention will now be further explained in the following examples. These examples are only intended to illustrate the invention and should in no way be considered to limit the scope of the invention.

Brief description of the drawings

In the examples below reference is made to the accompanying drawing on which:

Fig. 1 A shows the effect of interleukin-6 gene knock out in male mice on mean body weight at different ages. Fig. 1 B shows the physical appearance of IL-6 knock out male mice at 9-10 months of age. The photo shows representative body shapes of IL-6 -/- and IL-6 +/+ male mice. The computerized tomography (CT) shows transverse sections of the abdomen of representative IL-6 -/- and IL-6 +/+ male mice (C).

Fig. 2 A, B and C illustrates the effects of interleukin-6 gene knock out on mean body weight at different ages in female mice (Fig. 2 A) and the effect of interleukin-6 gene knock out on mean body mass index (Fig. 2 B) (BMI, body weight/(crown-rump length)^2) and mean visceral transversal width (mm) (Fig. 2 C) were also investigated in 9 month-old female mice.

Fig 3. Shows the measured daily food intake during three consecutive days in 11 month-old female IL-6 +/+ and IL-6 -/- mice.

Fig. 4 A, B and C illustrates the effects of interleukin-6 gene knock out in female mice on serum triglyceride levels (Fig. 4 A), serum cholesterol (Fig. 4 B), and serum leptin levels (Fig 4 C).

Examples

The IL-6 knock out mice (i.e. IL-6 -/- mice) and the corresponding controls used in these examples were kindly provided by Dr. Manfred Kopf at Basel Institute of Immu-

nology, Basle, Switzerland (see e.g. Kopf, M. (1994), supra). The IL-6 -/- mice were back crossed 7-8 times with c57Bl/6 mice to gain a strain of mice genetically consisting of more than 95 % c57Bl/6. As controls to the IL-6 -/- mice, wild type c57Bl/6 mice (i.e. IL-6 +/+ mice) (Bomholtgård Breeding & Research Centre A/S) were used. The mice were kept at standardized conditions with standard low fat chow and water freely available. The body weight of the IL-6 -/- mice and wild type control female mice were recorded regularly. Food intake was measured keeping two female mice per cage. The amount of chow was recorded once per day. The crown-rump length and the transversal abdominal diameter were measured in anesthetized animals by dual x-ray absorptiometry (DEXA) using the Norland pDEXA Sabre (Fort Atkinson, WI, USA). Body mass index was then calculated for each mouse as body weight/crown-rump length^2. Visceral and subcutaneous obesity was also evaluated by computerized tomography (CT) at a level 5 mm cranially of the junction between the L6 and S1 vertebras. Serum triglyceride and serum cholesterol levels were measured by standard clinical methods. Mouse leptin in serum was measured using a kit from Linco Res. (St Charles, Mo, USA) as described (see Ahrén, B. et al. (1997) Regulation of plasma leptin in mice: Influence of age, high-fat diet and fasting, Am. J. Physiol. 273, R113-R120).

Differences between IL-6 -/- and IL-6 +/+ control mice were determined by Student's t-test. When more than two groups were compared, statistics were calculated by one-way ANOVA followed by Student-Newman-Keuls multiple range test.

<u>Example 1</u>

IL-6 -/- knock-out male mice were not heavier than their wild type littermates at 2-5 months of age. However, the body weight of 9 months old IL-6 -/- male mice was higher than that of the corresponding wild type ani-

mals, as evident from Fig. 1 A. The physical appearance of male mice at 9-10 months of age clearly showed that the IL-6 -/- mouse was considerably fatter than a wild type control of the same age, as shown in Fig. 1 B). Computerized tomography (CT) of the abdomen clearly indicated that both visceral (intraabdominal) and subcutaneous fat mass were markedly increased in the IL-6 -/- mice compared to the wild type control, as evident from Fig. 1 C.

Example 2

In this example the effects of IL-6 knock-out on body weight was studied at different ages in female mice. The body weight did not differ between wild type and knock-out female mice between two and five months of age, but between seven and nine months of age the body weight was significantly higher in IL-6 -/- than in wild type +/+ mice, as seen in Fig. 2 A. The body mass index of 9-10 months old IL-6 knock-out female mice was higher than that of the corresponding wild type females, which is illustrated in Fig. 2B. The transversal abdominal diameter, as measured by DEXA, was also larger in IL-6 knock-out female mice than in wild type controls at 9-10 months of age (Fig. 2C).

Example 3

Thereafter the daily food intake for three consecutive days was studied for 11 months old IL-6 -/- female mice compared to in wild type IL-6 +/+ controls. From the results shown in Fig. 3 it is clearly evident that the food intake was increased in the L-6 -/- mice compared to the controls.

Example 4

Serum triglyceride and cholesterol levels of 11 months old female IL-6 -/- mice were compared to wild type IL-6 +/+ controls. As can be seen in Fig. 4 A and

4 B the serum triglyceride and cholesterol levels were considerably higher in the IL-6 -/- mice. Also the circulating levels of leptin were markedly higher, i.e., about three times, compared to those of wild type mice, as seen in Fig. 4 C.

CLAIMS

1. Use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for the production of a medicinal product for treatment of obesity and/or disturbances of blood fats.

2. Use according to claim 1, wherein said substance is an IL-6 receptor agonist.

3. Use according to claim 2, wherein said substance is IL-6.

4. Use according to any one of the claims 1-3, wherein said obesity and/or disturbances of blood fats is caused by a pathological disturbance of fat metabolism.

5. Use according to claim 4, wherein said obesity is mainly visceral or intraabdominal.

6. Use according to any one of the claims 1-5, wherein said obesity is observed despite high levels of circulating leptin.

7. Use according to any one of the claims 1-6, wherein said obesity is accompanied by leptin insensitivity.

8. Use according to any one of the claims 1-3, wherein said obesity and/or disturbances of blood fats is caused by a pathological disturbance of blood fats.

9. Use according to claim 8, wherein said condition is a pathological increase of serum triglycerides.

10. Use according to claim 8, wherein said condition is a pathological increase of serum cholesterol.

11. Use according to any one of the claims 1-10, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

12. Use according to any one of the claims 1-11, wherein said medicinal product is suitable for treatment of a condition due to ageing.

13. Use according to claim 12, intended for a human patient of the age 30 years or older.

14. Use according to any one of the claims 1-13, wherein said medicinal product further comprises a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist.

15. Use according to claim 14, wherein said factor is a factor acting via gp130.

16. Use according to claim 14, wherein said factor is leptin.

17. A method for treatment of obesity and/or disturbances of blood fats wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient.

18. A method according to claim 17, wherein said substance is an IL-6 receptor agonist.

19. A method according to claim 18, wherein said substance is IL-6.

20. A method according to any one of the claims 17-19, wherein said obesity and/or disturbances of blood fats is caused by a pathological disturbance of fat metabolism.

21. A method according to claim 20, wherein said obesity is mainly visceral or intraabdominal.

22. A method according to according to any one of the claims 17-21, wherein said obesity is observed despite high levels of circulating leptin.

23. A method according to according to any one of the claims 17-22, wherein said obesity is accompanied by leptin insensitivity.

24. A method according to any one of the claims 17-19, wherein said obesity and/or disturbances of blood fats is caused by a pathological disturbance of blood fats.

25. A method according to claim 24, wherein said condition is a pathological increase of serum triglycerides.

26. A method according to claim 24, wherein said condition is a pathological increase of serum cholesterol.

27. A method according to any one of the claims 17-26, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

28. A method according to any one of the claims 17-27, wherein said medicinal product is suitable for treatment of a condition due to ageing.

29. A method according to claim 28, wherein said patient is a human of the age 30 years or older.

30. A method according to any one of the claims 17-29, wherein said IL-6 receptor agonist is administered in combination with a factor that will intensify the effect of said IL-6 receptor agonist.

31. A method according to claim 30, wherein said factor is a factor acting via gp130.

32. A method according to claim 30, wherein said factor is leptin.

ABSTRACT

Use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist, preferably IL-6, for the production of a medicinal product for treatment of obesity and/or disturbances of blood fats is disclosed.

Also a method for treatment of obesity and/or disturbances of blood fats wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 receptor agonist is administered is disclosed.

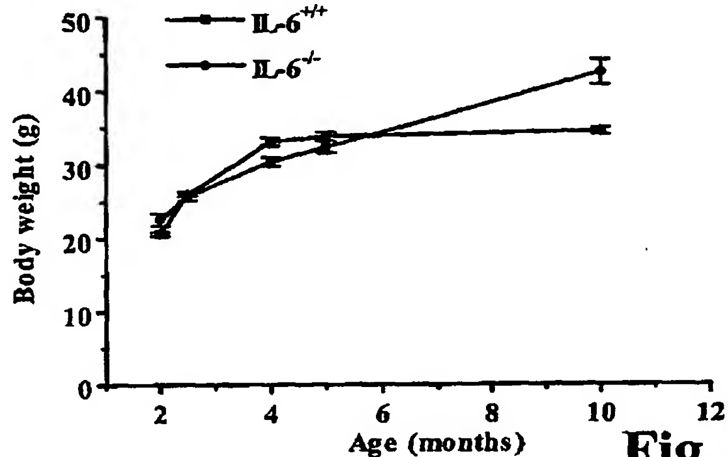

Fig. 1 A

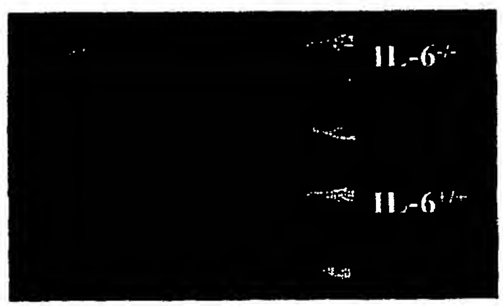

Fig. 1 B

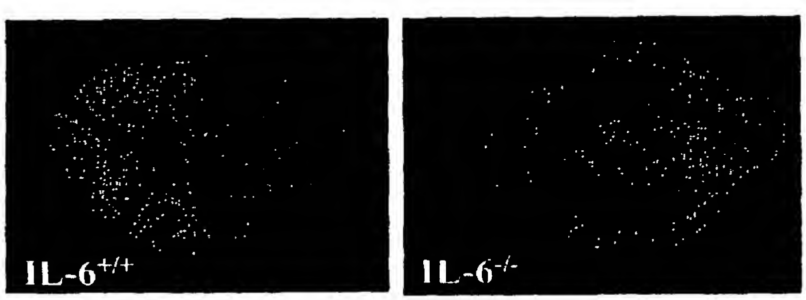

Fig. 1 C

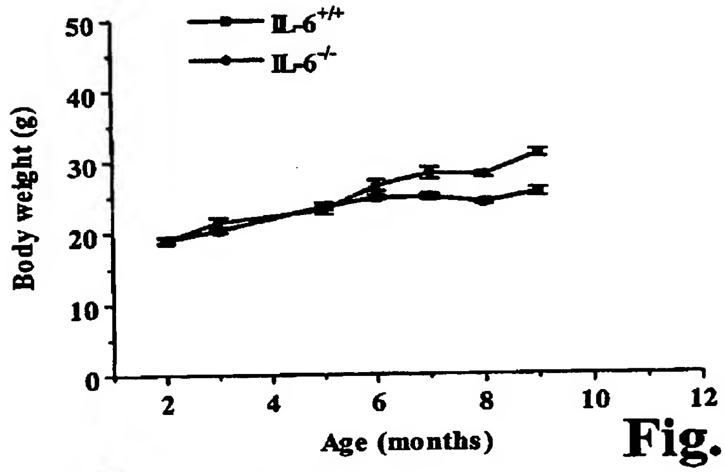

Fig. 2 A

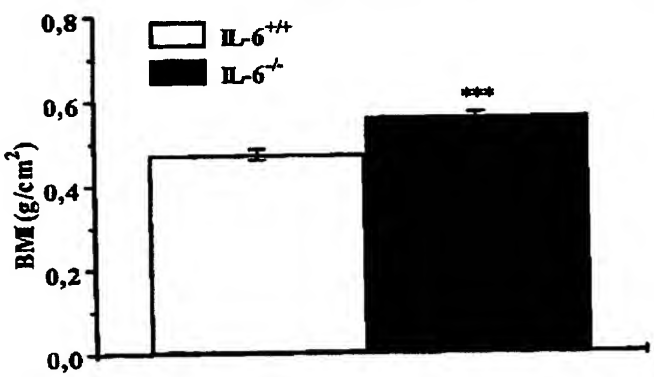

Fig. 2 B

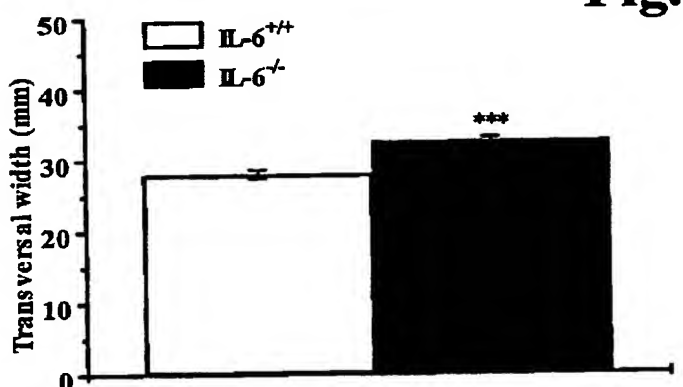

Fig. 2 C

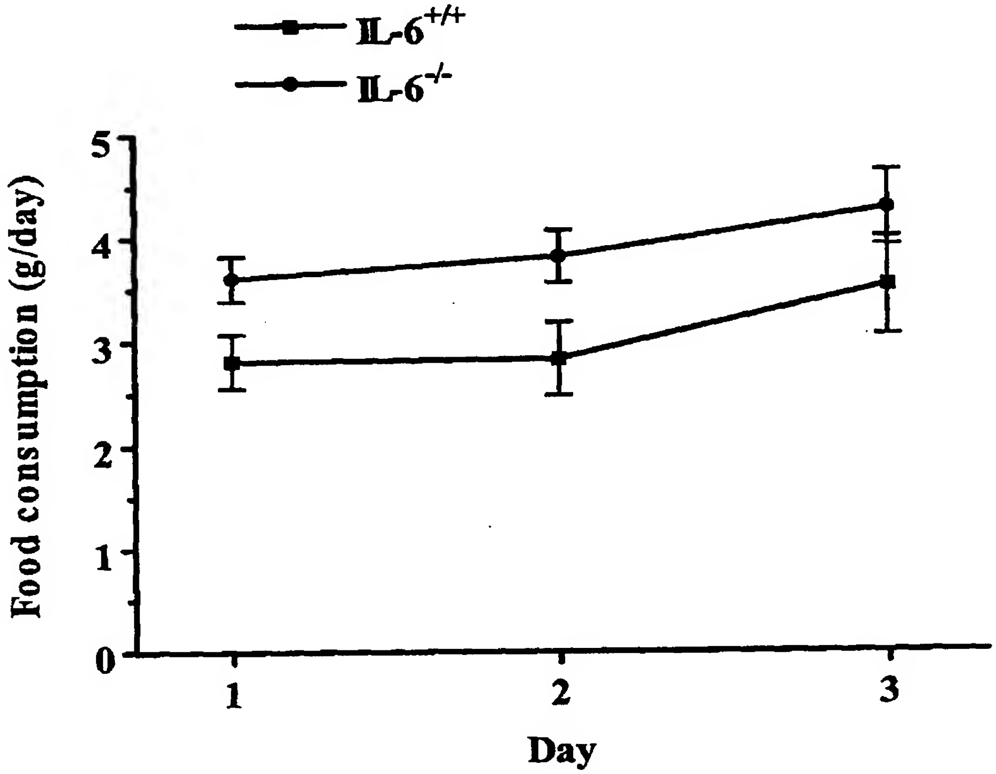

Fig. 3

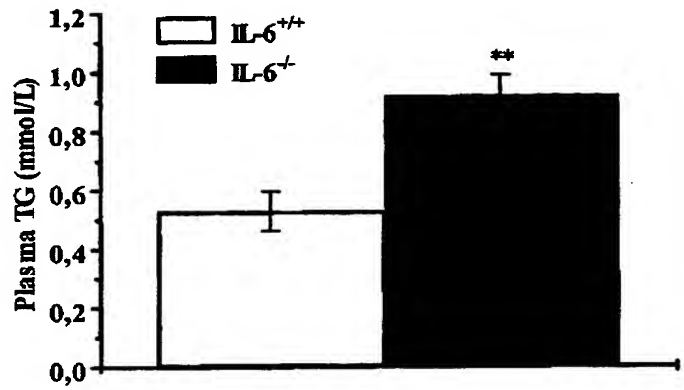

Fig. 4 A

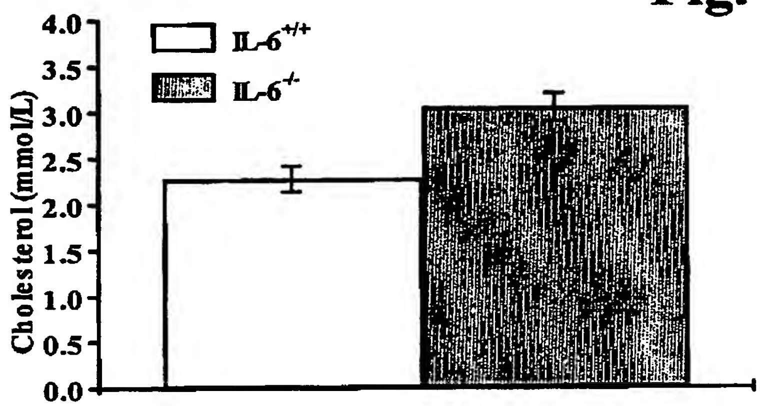

Fig. 4 B

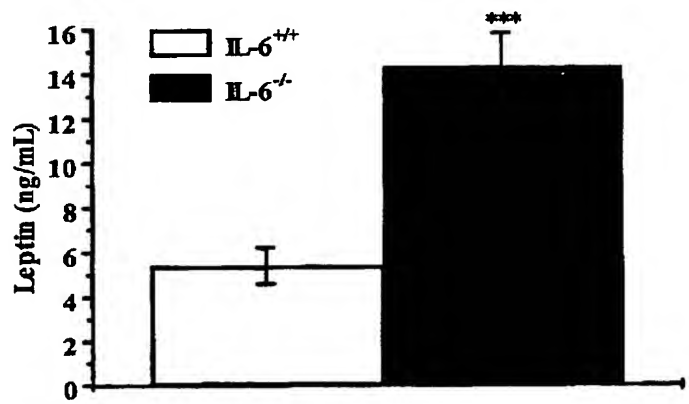

Fig. 4 C